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Biocatalysis in ionic liquid: degradation of phenol by laccase

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Abstract

The aim of this work was to optimize the reaction conditions for the degradation of phenol in aqueous and ionic liquid (IL)-aqueous solution by biocatalysis using two commercial laccases. In order to evaluate the effect of IL on laccase-mediated reactions, the IL 1-ethyl-3-methylimidazolium ethylsulfate, [C_2mim][$EtSO_4$] was selected as reaction media for this study. Preliminary studies for phenol degradation were carried out in a lab-scale stirred batch reactor with 2 commercial laccases (from *Trametes versicolor*; Sigma and from *Aspergillus*; Novozymes). The synthetic effluent was prepared with phenol in appropriated buffer solutions, with pH varying from 3.0 to 9.0 and different enzyme mediators: 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), N-hydroxyacetanilide (NHA) and (2,2,6,6-tetramethylpiperidin-1-yloxy) (TEMPO). For the optimization of reaction conditions, laccase concentration was varied in the range 1000-2000 U/L, pH in the range 6.0-7.0 and IL concentration in the range 10-20% v/v. For comparison, acetonitrile was used as organic solvents at the same conditions. Quantitative analysis of phenols and by-products formed were determined by HPLC (LaChrom Elite HPLC) with a column RP-18 LichroCART at 25°C. In conclusion, laccase (from the class of oxidases) was shown to possess catalytic activity for the degradation of phenol in systems containing ionic liquids. This finding opens up promising perspectives for applying these environmentally benign solvents to a broad range of important oxidative biotransformations.

Keywords: Laccase; ionic liquid; biocatalytic reaction; phenol

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1. Introduction

Industrial processes generate a variety of molecules that may pollute air, soil and water with negative impacts for ecosystems and humans (toxicity, carcinogenic and mutagenic properties). Due to this problem, research has been conducted to investigate the new possibilities of applying enzymes for organic waste treatment. It is known that several types of industrial and agricultural wastes contain phenolic compounds: paints, pesticides, wastes from coal conversion, polymeric resins and petroleum. Due to the fact that the classic treatments of industrial effluents containing phenolic compounds have not yet been able to solve all the associated problems involved or present high costs of process, there is a great need to develop an economical, effective and innovative way for the treatment of these wastes [1]. The potential advantages of the enzymatic treatment when compared to conventional process include: operation at high and low contaminant concentrations; operation over a wide range of pH and temperature. The use of enzymes has been standard in several industries for many years. Only recently they have been studied as a means for enhancing bioremediation. Enzymes possess the ability to break down bonds within organic compounds and/or catalyze their transformation into less toxic and more biodegradable forms. Different studies show that ligninolytic enzymes (laccases) can degrade a wide variety of recalcitrant compounds. Furthermore, the number of laccase substrates can be extended by addition of a redox mediator [2].

Currently, the enzymatic oxidation of phenolic compounds with poor water solubility by enzymatic catalysis is carried out in nonaqueous systems, and the use of organic solvents is required to dissolve them. As disadvantages, the molecules of solvent may directly interact with the enzymes, thus changing their structure, causing irreversible inactivation of the enzymes. Most solvents used present toxicity and low biodegradability. In order to develop a green biotechnological solution, the use of Ionic Liquids (ILs) as solvent or co-solvent is an alternative extremely necessary and interesting [3]. ILs are defined as substances composed only by ions which are liquid at room temperature. Among their properties, publications usually highlight their negligible vapor pressure, thermal and chemical stability, and the possibility to “design” their physicochemical properties by the suitable choice of the anion and the cation [4]. Because of their negligible vapor pressure, ILs are claimed as “green” alternatives for volatile organic compounds (VOCs). Moreover, several ILs have demonstrated to be adequate solvents for enzyme reactions [5].

2. Materials and Methods

2.1 Chemicals

Laccases from *Trametes versicolor* and from *Aspergillus* were purchased from Sigma and kindly provided by Novozymes (Denmark), respectively. Phenol 99% was purchased from Merck. 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), N-hydroxyacetanilide (NHA) or (2,2,6,6-tetramethylpiperidin-1-yloxy) (TEMPO) were obtained from Sigma-Aldrich. The IL 1-ethyl-3-methylimidazolium ethylsulfate, $[C_2mim][EtSO_4]$ was synthetized in our laboratory, following a procedure available in the literature [6].

2.2 Degradation of phenol catalyzed by laccase in aqueous solution

2.2.1 Mediator screening

The reaction mixture for mediator screening consisted of an aqueous solution of phenol (50 mg/L), laccase, redox mediator ABTS, NHA and TEMPO (0.1 mM) and 50 mM phosphate buffer in a final volume of 25 ml. The reactions were incubated at 25ºC with stirring during one day (necessary time to reach the equilibrium). Control without mediator and control without laccase were carried out in parallel.

2.2.2 Effect of pH

To study the effect of pH on degradation of phenol (50 mg/L), 0.075 mM of ABTS as mediator and commercial laccase from *T. versicolor* were incubated in 25 mL Erlenmeyer flasks under stirring during one day.

2.3 Degradation of phenol catalyzed by laccase in ionic liquid media

For the optimization of laccase concentration in IL [C_2mim][$EtSO_4$] the reaction mixture was prepared with 1000-2000 U/L of enzyme concentration, pH 6.0-7.0 and IL concentration 10-20% v/v. For comparison, acetonitrile was used as organic solvents at the same conditions. Quantitative analysis of phenols and by-products formed were determined by HPLC system (LaChrom Elite HPLC) with a column RP-18 LichroCART at 25°C.

3. 3. Results and discussion

3.1 Phenol degradation in aqueous medium

Comparing both enzymes, laccase from *T. versicolor* showed a higher capacity for phenol degradation, three times, and also a higher rate of reaction than laccase from *Aspergillus*. Thus, this enzyme was used for all further experiments. In order to improve the oxidation of phenol by laccase, the presence of enzyme mediators is required. From the obtained results (Fig. 1), the ABTS mediator promoted the highest phenol degradation (91%), although presenting a slight difference when compared to the others mediators or laccase alone (degradation > 80%). Among the pH values tested (from 6.0 to 8.0), the highest phenol degradation was obtained for pH 6.0, an increase of two times, from 45% to 90% of phenol degradation for pHs 7.0 - 8.0 and pH 6.0, respectively.

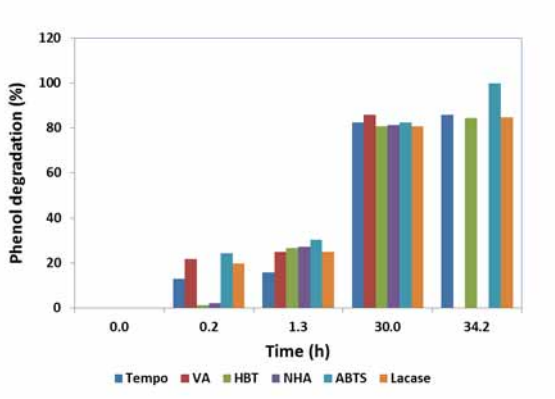

Fig. 1. Degradation of phenol by laccase using different mediators

3.2 Phenol degradation in ionic liquid medium

At the optimized conditions of laccase degradation in aqueous media, the reactions were carried out in the presence of the ionic liquid [C_2mim][$EtSO_4$]. The results presented in Table 1 show that the enzymatic conversions were lower than in aqueous media, however when compared to the organic solvent, acetonitrile, the phenol degradation was reduced. Results from literature show that laccase is stable in [C_2mim][$EtSO_4$] after one day using ABTS as substrate [5]. It could be suggested that different substrates give different effects on the catalytic properties of laccase. In this more complex reaction media, laccase was inhibited by the presence of the IL. It is still unclear regarding what characteristics of organic solvents that can affect the enzymatic activity however the solvent's hydrophobicity is an indicator for predicting the degree of enzymatic activity in organic media [7].

Table 1. Optimization of pH, laccase concentration (U/L) and solvent concentration (%) for the phenol degradation. The reactions were carried in the presence of ionic liquid or acetonitrile

Solvent (%)	pH	Laccase (U/L)	Phenol degradation (%)	
			In ionic liquid	In acetonitrile
10	6	1000	26.4	57.7
20	6	1000	20.3	57.0
10	7	1000	16.3	54.5
20	7	1000	12.4	50.7
10	6	2000	25.9	53.9
20	6	2000	24.7	15.1
10	7	2000	25.4	58.0
20	7	2000	13.8	60.2

In conclusion, laccase (from the class of oxidases) possesses catalytic activity for the degradation of phenol in aqueous systems. The phenol degradation in ionic liquid media presented a low degradation of phenol and future work must be done in order to increase the degradation. This finding starts to open

promising perspectives for applying these environmentally benign solvent to a broad range of important oxidative biotransformations.

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